

beta-gal DNA using LipofectAMINE™ reagent. The transfection medium was composed of 0.8 ml DMEM with or without Polybrene™ and 5% FBS. The day following transfection, the cells were rinsed with PBS, fixed and stained with X-gal. Peak activity points from the dose-response were photographed.

Group	LipofectAMINE™ (μl)	Polybrene™ (μg/ml final)	FBS
A	5	—	—
B	8	—	+
C	12	80	+

The optimal conditions from dose responses of LipofectAMINE™ and Polybrene™ in transfections with or without 5% FBS are as follows: With BHK21 (1×10^5), 8 μl of LipofectAMINE™ was used in the absence of FBS and Polybrene™; 9 μl of LipofectAMINE™ was used in the presence of FBS and the absence of Polybrene™, and 15 μl of LipofectAMINE™ was used in the presence of both FBS and Polybrene™. Polybrene™ was used at 20 μg/ml. The level of transfection (number of stained cells) in the presence of serum and Polybrene™ was nearly equivalent to that seen in serum-free conditions, however more cells survived (less toxicity), so the percent transfected cells is not as high.

With the COS-7 cells (4×10^4), 5 μl of LipofectAMINE™ was used in the absence of FBS and Polybrene™; 5 μl of LipofectAMINE™ was used in the presence of FBS and the absence of Polybrene™, and 12 μl of LipofectAMINE™ was used in the presence of both FBS and Polybrene™. Polybrene™ was used at 80 μg/ml. These cells showed less toxicity in response to serum-free LipofectAMINE™ transfection. Once again, the number of stained cells in the presence of serum and Polybrene™ is nearly equivalent to that seen in the serum-free transfection.

Example 2

The activity of β-galactosidase expression was determined from dose-response transfection experiments as described in Example 1. 1×10^5 BHK-21 cells were plated per 35 mm well (6-well plate). The day after plating, cells were rinsed with DMEM and fed 0.8 ml of DMEM with or without polybrene™ and 5% FBS. The cells were transfected with 1 μg pCMV β-gal DNA and LipofectAMINE™ Reagent. The day after transfection, cells were rinsed with PBS, harvested and assayed for β-galactosidase activity. Peak activity points from the dose-response were graphed as shown in FIG. 1.

The results at optimal conditions are as follows: At optimal LipofectAMINE™ concentration in the presence of 5% FBS, BHK-21 and COS-7 transfections showed a 20–30-fold increase in total activity of β-galactosidase when Polybrene™ was present in the transfection medium. The amount of LipofectAMINE™ required for peak activity in serum and Polybrene™ was higher than that required in serum-free medium.

Group	LipofectAMINE™ (μl)	Polybrene™ (μg/ml final)	FBS
A	6	—	—
B	6	—	+
C	18	20	+

4×10^4 COS-7 cells were plated in 24-well plates. The day after plating, cells were transfected with 0.4 μg pCMV

beta-gal DNA using LipofectAMINE™ reagent. The transfection medium was composed of 0.8 ml DMEM with or without Polybrene™ and 5% FBS. The day following transfection, the cells were rinsed with PBS, harvested and assayed for β-galactosidase activity. Peak activity points from the dose-response were graphed as shown in FIG. 2.

Group	LipofectAMINE™ (μl)	Polybrene™ (μg/ml final)	FBS
A	5	—	—
B	8	—	+
C	12	80	+

When LipofectAMINE™ is used in transfections in the presence of serum, activity is much lower than without serum, and the peak position for LipofectAMINE™ concentration shifts. When the serum-containing medium is supplemented with 20–40 μg per ml of Polybrene™, activity is greatly enhanced.

As will be appreciated by those skilled in the art, the foregoing examples are illustrative only and not meant to limit the scope of this invention which is defined by the appended claims and by equivalents to the claimed embodiments which would be obvious to one skilled in the art in view of the teachings herein.

We claim:

1. In a method of transfecting an animal cell in the presence of serum, comprising contacting said cell with a lipid aggregate comprising nucleic acid and a cationic lipid, wherein the improvement comprises: contacting said cell with said lipid aggregate in the presence of a polycationic compound, thereby transfecting said animal cell with said nucleic acid.

2. In a method of transfecting an animal cell in the presence of serum, comprising contacting said cell with a lipid aggregate comprising nucleic acid and a cationic lipid, wherein the improvement comprises: either (a) first contacting said cell with a polycationic compound to form a complex of said cell with said polycationic compound followed by contacting said cell complex with said lipid aggregate; or (b) first contacting said lipid aggregate with said polycation compound to form a mixture followed by contacting said cell with said mixture, thereby transfecting said animal cell with said nucleic acid.

3. A method of claim 2 wherein said polycationic compound is POLYBRENE™.

4. A method of claim 2 wherein said lipid aggregate comprises a compound toxic to said cells.

5. A method of claim 2 wherein said cells are mammalian cells.

6. A method of claim 2 wherein said serum is fetal bovine serum.

7. A method of claim 2 wherein said serum is human serum.

8. A method of claim 2 wherein said lipid aggregate comprises LipofectAMINE™.

9. A method of claim 2 wherein said lipid aggregate comprises Lipofectin™.

10. A method of claim 2 wherein said lipid aggregate comprises LipofectACE™.

11. A method of claim 2 wherein said nucleic acid is DNA.

* * * * *